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JC09 PCT/PTO 11 DEC 2001 PCT

PTO/SB/64/PCT (10-00)

Approved for use through 10/31/2002. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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**PETITION FOR REVIVAL OF AN INTERNATIONAL APPLICATION FOR PATENT  
DESIGNATING THE U.S. ABANDONED UNINTENTIONALLY UNDER 37 CFR 1.137(b)**

Docket Number (Optional)

5091US

First named inventor: Jan Dirk VAN DER KLIS

International (PCT) Application No.: PCT/NL00/00199 U.S. Application No.:  
(if known)

Filed: 24 March 2000

Title: INCREASING PLASMA CALCITRIOL LEVELS

Attention: PCT Legal Staff  
Box PCT  
Assistant Commissioner for Patents  
Washington, D.C. 20231

The above-identified application became abandoned as to the United States because the fees and documents required by 35 U.S.C. 371(c) were not filed prior to the expiration of the time set in 37 CFR 1.494(b) or (c) or 1.495(b) or (c) as applicable. The date of abandonment is the day after the date on which the 35 U.S.C. 371(c) requirements were due. See 37 CFR 1.494(g) or 1.495(h).

**APPLICANT HEREBY PETITIONS FOR REVIVAL OF THIS APPLICATION**

NOTE: A grantable petition requires the following items:

- (1) Petition fee
- (2) Proper reply
- (3) Terminal disclaimer with disclaimer fee--required for all international applications having an international filing date before June 8, 1995; and
- (4) Statement that the entire delay was unintentional.

**1. Petition fee**

☐ Small entity - fee \$ \_\_\_\_\_ (37 CFR 1.17(m)). Applicant claims small entity status.  
See 37 CFR 1.27.

☒ Other than small entity - fee \$ 1,280 (37 CFR 1.17(m))

**2. Proper reply**

A. The proper reply (the missing 35 U.S.C. 371(c) requirement(s) in the form of  
Filing under 35 U.S.C. 371 (identify type of reply):

- ☐ has been filed previously on \_\_\_\_\_
- ☒ is enclosed herewith.

12/27/2001 HNGUYEN 00000065 PCT/NL00/00199

01 FC:141 1280.00 OP

07/03/2002 LLANDGRA 00000001 10018390

01 FC:141

1280.00 OP

[Page 1 of 2]

Burden Hour Statement: This form is estimated to take 1.0 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Adjustment date: 07/03/2002 LLANDGRA  
12/27/2001 HNGUYEN 00000065 PCT/NL00/00199  
01 FC:141 1280.00 OP

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

### 3. Terminal disclaimer with disclaimer fee

- ☒ Since this international application has an international filing date on or after June 8, 1995, no terminal disclaimer is required.
- ☐ A terminal disclaimer (and disclaimer fee (37 CFR 1.20(d)) of \$\_\_\_\_\_ for a small entity or \$\_\_\_\_\_ for other than a small entity) disclaiming the required period of time is enclosed herewith (see PTO/SB/63).

4. Statement. The entire delay in filing the required reply from the due date for the required reply until the filing of a grantable petition under 37 CFR 1.137(b) was unintentional.

**WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.**

11 December 2001  
Date

all @ 1m

Signature

Telephone  
Number: (801) 532-1922

Allen C. Turner

Typed or printed name

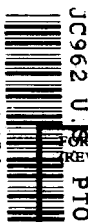
P. O. Box 2550

Address

Salt Lake City, Utah 84110

Enclosures: ☒ Response  
☒ Fee Payment  
☐ Terminal Disclaimer Form  
☐

12/11/01



12-19-01

10/018390.090907 PCT

JC09 Rec'd PCT/PTO 11 DEC 2001

FORM PTO-1390  
REV. 11-2000

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

5091US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

10/018390

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

INTERNATIONAL APPLICATION NO.

PCT/NL00/00199

INTERNATIONAL FILING DATE

24 March 2000 (24.03.00)

PRIORITY DATE CLAIMED

24 March 1999 (24.03.99)

TITLE OF INVENTION

INCREASING PLASMA CALCITRIOL LEVELS

APPLICANT(S) FOR DO/EO/US

Jan Dirk VAN DER KLIS, Cornelis Willem SCHEELE

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☐ is attached hereto.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☒ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:

Petition for Revival  
Copy of Form PCT/IB/308  
Preliminary Examination Report  
International Application

## NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL740517052US  
Date of Deposit with USPS: December 11, 2001  
Person making Deposit: Orlena Howell

U.S. APPLICATION NO. <b>10/018390</b> INTERNATIONAL APPLICATION NO. PCT/NL00/00199		ATTORNEY'S DOCKET NUMBER <b>5091US</b>					
21. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... <b>\$1000.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... <b>\$860.00</b>  International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b>  International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b>  <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>		<b>CALCULATIONS PTO USE ONLY</b>          <table style="width:100%; border: none;"> <tr> <td style="width: 50%; border: none;">\$ 860.00</td> <td style="width: 50%; border: none;"></td> </tr> <tr> <td style="border: none;">\$ 130.00</td> <td style="border: none;"></td> </tr> </table>		\$ 860.00		\$ 130.00	
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Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		<table style="width:100%; border: none;"> <tr> <td style="width: 50%; border: none;">\$</td> <td style="width: 50%; border: none;"></td> </tr> <tr> <td style="border: none;">\$</td> <td style="border: none;"></td> </tr> </table>		\$		\$	
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CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE				
Total claims	21 - 20 =	1	x \$18.00				
Independent claims	6 - 3 =	3	x \$84.00				
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00				
<b>TOTAL OF ABOVE CALCULATIONS =</b>			\$ 1,260.00				
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			\$ +				
<b>SUBTOTAL =</b>			\$ 1,260.00				
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			\$				
<b>TOTAL NATIONAL FEE =</b>			\$ 1,260.00				
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property +			\$				
<b>TOTAL FEES ENCLOSED =</b>			\$ 1,260.00				
<b>Amount to be refunded:</b>			\$				
<b>charged:</b>			\$				

a. ☒ A check in the amount of \$ 1,260.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_ to cover the above fees.  
 A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any  
 overpayment to Deposit Account No. 20-1469. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card**  
**information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR  
 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Allen C. Turner  
 TRASKBRITT, P.C.  
 P. O. Box 2550  
 Salt Lake City, Utah 84110

*Allen C. Turner*  
 SIGNATURE  
 Allen C. Turner  
 NAME  
 33,041  
 REGISTRATION NUMBER

10018390 10/018390

JC13 Rec'd PCT/PTO 1.1 DEC 2001

*Pre Amndt  
4/A*

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:**

Van Der Klis et al.

**Serial No.:** To be assigned

**Filed:** October 17, 2001

**For:** INCREASING CALCITRIOL LEVELS

**Examiner:** To be assigned

**Group Art Unit:** To be assigned

**Attorney Docket No.:** 5091US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL740517052US

Date of Deposit with USPS: December 11, 2001

Person making Deposit: Orlena Howell

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please amend the above identified patent application as follows:

**IN THE CLAIMS:**

Please amend the claims to appear as follows. Please note that all claims pending in the application are presented here in clean form for clarity and a marked up version of the claim amendments is attached.

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- A
1. (Amended) A method for increasing immune competence of an animal, said method comprising inducing calcitriol synthesis in said animal wherein said animal is selected from a group of poultry or pigs.
  2. (Amended) The method of claim 1 further comprising feeding said animal a food capable of inducing calcitriol synthesis.
  3. (Amended) The method of claim 2 wherein said food is supplemented or enriched with a substance capable of inducing calcitriol synthesis.
  4. (Amended) The method of claim 3 wherein said substance comprises a phyto-estrogen.
  5. (Amended) The method of claim 3 wherein said substance is derived from a leguminous seed.
  6. (Amended) The method of claim 5 wherein said leguminous seed is soy.
  7. (Amended) The method of claim 1 wherein also phytate degradation capacity of said animal is increased.
  8. (Amended) A method for preparing pig or poultry feed for increasing immune competence and/or phytate degradation capacity of pigs or poultry comprising supplementing or enriching a pig or poultry feed with a substance capable of inducing calcitriol synthesis.

9. (Amended) The method of claim 8, wherein said substance comprises a phyto-  
estrogen.

10. (Amended) The method of claim 9, wherein said substance is derived from a  
leguminous seed.

11. (Amended) The method of claim 10, wherein said leguminous seed is soy.

---

Please cancel claim 12, without prejudice or disclaimer.

---

13. (Amended) A supplement for an animal food suitable for increasing immune  
competence and/or phytate degradation capacity of an animal comprising a substance capable of  
inducing calcitriol synthesis.

14. (Amended) The supplement of claim 13, wherein said substance comprises a phyto-  
estrogen.

15. (Amended) An animal food comprising a supplement of claim 13.

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16. A pig or poultry food enriched for a substance capable of inducing calcitriol  
synthesis.

Please cancel claim 17, without prejudice or disclaimer.

---

18. (Amended) A method for increasing immune competence and/or phytate degradation  
of an animal selected from the group consisting of pig and poultry comprising feeding said animal  
a food according to claim 16.

---

Please cancel claims 19, 20 and 21, without prejudice or disclaimer.

---

Please add the following new claims:

---

22. (New) A method of reducing the dietary intake of antibiotics of an animal comprising  
administering to said animal a food comprising a substance capable of inducing calcitriol synthesis.

23. (New) The method of claim 22, wherein said substance comprises a phyto-estrogen.

24. (New) The method of claim 23, wherein said substance is derived from a leguminous seed.

25. (New) The method of claim 24, wherein said seed is soy.

---



**REMARKS**

The Office is respectfully requested to enter the above amendments prior to the calculation of the filing fee in this application. Should the Office determine that additional issues remain which might be resolved by a telephone conference, it is respectfully invited to contact applicants' undersigned attorney.

Respectfully Submitted,



Bretton L. Crockett  
Registration Number 44,632  
Attorney for Applicants  
TRASKBRITT, PC  
P.O. Box 2550  
Salt Lake City, Utah 84110  
Telephone: (801) 532-1922

Date: May 17, 2001

ACT/BLC

N:\2183\5091\5091 Preliminary Amendment.wpd

**VERSION WITH MARKINGS TO SHOW CHANGES MADE****IN THE CLAIMS:**

Please amend the claims as follows:

1. (Amended) A method for increasing immune competence of an animal, said method comprising inducing calcitriol synthesis [by] in said animal wherein said animal is selected from [the] a group of poultry or pigs.
2. (Amended) [A] The method [according to] of claim 1 further comprising feeding said animal a food capable of inducing calcitriol synthesis.
3. (Amended) [A] The method [according to] of claim 2 wherein said food is supplemented or enriched with a substance capable of inducing calcitriol synthesis.
4. (Amended) [A] The method [according to] of claim 3 wherein said substance comprises a phyto-estrogen.
5. (Amended) [A] The method [according to] of claim 3 [or 4] wherein said substance is derived from a leguminous seed.
6. (Amended) [A] The method [according to] of claim 5 wherein said leguminous seed is soy.
7. (Amended) [A] The method [according to anyone of the foregoing claims whereby] of claim 1 wherein also phytate degradation capacity of said animal is increased.
8. (Amended) A method for preparing pig or poultry [food suitable for] feed for increasing immune competence and/or phytate degradation capacity of pigs or poultry comprising supplementing or enriching [said food] a pig or poultry feed with a substance capable of inducing calcitriol synthesis.

9. (Amended) [A] The method [according to] of claim 8, wherein said substance comprises a phyto-estrogen.

10. (Amended) [A] The method [according to] of claim 9, wherein said substance is derived from a leguminous seed.

11. (Amended) [A] The method [according to] of claim 10, wherein said leguminous seed is soy.

Please cancel claim 12, without prejudice or disclaimer.

13. (Amended) A supplement for an animal food suitable for increasing immune competence and/or phytate degradation capacity of [said] an animal comprising a substance capable of inducing calcitriol synthesis.

14. (Amended) [A] The supplement [according to] of claim 13, wherein said substance comprises a phyto-estrogen.

15. (Amended) An animal food comprising a supplement of claim 13 [or 14].

Please cancel claim 17, without prejudice or disclaimer.

18. (Amended) A method for increasing immune competence and/or phytate degradation of [a] an animal selected from the group consisting of pig [or] and poultry comprising feeding said animal a food according to [anyone of claims 16 to 18] claim 16.

Please cancel claims 19, 20 and 21, without prejudice or disclaimer.

Please add the following new claims:

22. (New) A method of reducing the dietary intake of antibiotics of an animal comprising  
administering to said animal a food comprising a substance capable of inducing calcitriol synthesis.
23. (New) The method of claim 22, wherein said substance comprises a phyto-estrogen.
24. (New) The method of claim 23, wherein said substance is derived from a leguminous seed.
25. (New) The method of claim 24, wherein said seed is soy.

21 05 2001

AMENDED CLAIMS

(34)

- Sub A1
1. A method for increasing immune competence of an animal comprising inducing calcitriol synthesis by said animal wherein said animal is selected from the group of poultry or pigs.
  2. A method according to claim 1 comprising feeding said animal a food capable of inducing calcitriol synthesis.
  3. A method according to claim 2 wherein said food is supplemented or enriched with a substance capable of inducing calcitriol synthesis.
  4. A method according to claim 3 wherein said substance comprises a phyto-estrogen.
  5. A method according to claim 3 or 4 wherein said substance is derived from a leguminous seed.
  6. A method according to claim 5 wherein said seed is soy.
  7. A method according to anyone of foregoing claims whereby also phytate degradation capacity of said animal is increased.
  8. A method for preparing a pig or poultry food suitable for increasing immune competence and/or phytate degradation capacity of pigs or poultry comprising supplementing or enriching said food with a substance capable of inducing calcitriol synthesis.
  9. A method according to claim 8 wherein said substance comprises a phyto-estrogen.
  10. A method according to claim 9 wherein said substance is derived from a leguminous seed.
  11. A method according to claim 10 wherein said seed is soy.
  12. Use of a dietary substance capable of inducing calcitriol synthesis for the preparation of a supplement for a pig or poultry food suitable for increasing immune competence and/or phytate degradation capacity of pigs or poultry.
  - Sub A2 13. A supplement for a pig or poultry food suitable for increasing immune competence and/or phytate degradation capacity of pigs or poultry comprising a substance capable of inducing calcitriol synthesis.
  14. A supplement according to claim 13 wherein said substance comprises a phyto-estrogen.
  15. A pig or poultry food comprising a supplement according to claim 13 or 14.

16. A pig or poultry food enriched for a substance capable of inducing calcitriol synthesis.
17. A method for increasing immune competence and/or phytate degradation of a pig or poultry comprising feeding said animal a food according to anyone of claims 1 to 18.
18. Use of a method according to claim 18 for reducing or abolishing dietary antibiotic intake by said animal.

Sub 13

ALL 4

## Title: INCREASING PLASMA CALCITRIOL LEVELS

The invention relates to animal food and supplements used therein.

In general, animal foods, such as composed feed (diets) for birds, fish and mammals, are composed from vegetable and/or animal sources to meet the requirements of these animals for essential nutrients and energy. For production (growth, eggs and milk) and maintenance (energy) animals require protein, carbohydrates, fats, minerals, and vitamins. According to the known dietary requirements of various animals different feed materials are mixed to compose complete diets which in general contain well known required nutrients and energy levels in a correct ratio.

In present animal husbandry, often selected strains of animals, such as ruminants, pigs, poultry and fish are used that are characterised by a high production level. Such modern strains are often obtained by genetic selection and require specific feed management. However, by genetically selecting animals for desired production traits, other traits of the animal, such as those relating to immune competence, have in general been neglected. All too often, these high producing animals are over-sensitive to disease caused by infections with micro-organisms such as bacteria because of a in general scant development of the animal's immune competence in relation to for example its fast growth rates or increased production of eggs or milk.

To accommodate for the in general low levels of immune competence observed in these selected animal strains, one often supplements animal food with compounds such as antibiotics, often euphemistically called "growth-additives". The preventive use of antibiotics in animal food is considered by many to be indispensable to avoid illness and death, low-production and economic loss.

However, adding antibiotics to animal food is now more and more considered to be very undesirable from the animal- and human-health perspective, firstly because of fears of the development of bacterial strains that have become resistant against the antibiotics used, secondly because of fears for the presence of residues of the dietary antibiotics used in products from food animals, such as meat, milk and eggs. Consequently, more and more consumers desire animal products that are residue free and are derived from animals fed no antibiotics.

Due to this consumer pressure, legislation relating to antibiotics use in animal food is now rapidly changing, for example, within five years from now the preventive use of antibiotics added to diets will likely be restricted or banned in EC countries. Especially the routine use of dietary antibiotics in foods for food animals (those animals producing goods for human consumption such as meat, eggs or milk) will be reduced or even abolished in the near future.

Therefor, alternative, and preferably natural, food products need be found to replace dietary antibiotics in animal food to guard health status and welfare of animals and prevent otherwise unavoidable losses in animal husbandry.

The invention provides a method for increasing immune competence of an animal by inducing calcitriol synthesis by said animal, for example by supplementing or enriching said animal's food with a substance capable of increasing plasma calcitriol levels. Naturally, calcitriol synthesis can also be increased by supplementing said animal's water with said substance, or by providing said substance orally, rectally or parenterally to said animal. Surprisingly it was found that by providing an animal with a food or substance that is capable to increase plasma calcitriol (or analogues or derivatives thereof) levels of said animal, it is possible to increase the immune competence of said animal (see for example figure 1).



Elevating calcitriol (1,25 dihydroxy-cholecalciferol, DHCC,  $1.25(\text{OH})_2\text{D}_3$ ) levels increase competence of the immune system in several ways. It, or its analogues or derivatives, stimulates phagocytic and antigen presenting activity, and thereby promotes initial immune responses. This involves among others activation of T-cells via antigen presentation, cytokine secretion and stimulation of specific antibody production by B-cells. These stimulatory effects are balanced by the ability of the hormone to inhibit T-cell proliferation, which is needed for the necessary feedback mechanisms regulating immune responses.

According to the invention it is possible to raise calcitriol plasma levels by dietary calcitriol supplementation, or by the addition of dietary supplements with precursors or analogues thereof, however, in a preferred embodiment, the invention provides a method for preparing an animal food suitable for increasing immune competence of said animal comprising supplementing or enriching said food with a substance capable of increasing plasma calcitriol levels wherein said substance or derivatives thereof induces calcitriol synthesis in said animal, thereby increasing calcitriol levels. Supplementing a food can for example be done by the addition of a fraction (such as the remains of soy when most fat and proteins have been removed) that is specifically rich in said substance that induces calcitriol synthesis in said animal, enrichment of a food in general occurs by depleting a food from a compound or substance, such as from fat or protein, that has not that inducing activity. Of course, enriched fractions can later be supplemented.

In a preferred embodiment a method according to the invention is provided wherein said inducing substance comprises a phyto-estrogen. Phyto-estrogens are estrogen-like compounds from plants, such as legumes and grains, with estrogenic activity. Phyto-estrogens are often divided into three classes: isoflavones, lignans and coumestrol. Especially soybeans contain high levels of isoflavones, of which the most abundant

are genistein (C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>) and daidzein (C<sub>15</sub>H<sub>10</sub>O<sub>4</sub>). Most isoflavones in plants are bound to sugars as glycosides (genistin and daidzin respectively). Grains and legumes are important sources of lignans, while coumestrol (C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>) generally is found in clover, alfalfa and peas. The relative activity of different phyto-estrogens can for example be found in Mayr et al, Toxicology 74:135-149; 1992. The content of phyto-estrogens in plants can vary considerably, for example the content of daidzein and genistein in soy depends upon breed, growing conditions, and year of harvest. In general the content of plasma calcitriol elevating compounds in whole plants, or even whole seeds, is too low, to raise plasma calcitriol levels sufficiently, and feeding enriched fractions or food supplemented with the desired compound would be desirable. Although phyto-estrogens are fat-soluble plant components, it is not likely that they are strictly localised in the fat fraction of the leguminous seeds. In plants isoflavones are bound to sugars (carbohydrate fraction) and we have observed that fat containing plant fractions, such as full fat soybeans, as well as fat-depleted plant fractions, such as solvent extracted soybean meal stimulate calcitriol levels in plasma.

In a preferred embodiment, a method according to the invention is provided wherein said substance is derived from a seed, preferably from a leguminous seed such as for example soy. It is provided herein for example that (fractionated) substances from leguminous seeds, such as soy beans, increase immune competence by increasing the levels of calcitriol in plasma of broiler chicks. Leguminous seeds themselves can be used as supplement in animal feed, however, due to the low concentration of bioactive compounds in these seeds, adding a bulk amount of leguminous seeds to animal feed would in general disturb the desired constitution of animal feed. Therefore, it is preferred to supplement animal food with a substance derived from specific fractions of plants, such as leguminous seeds, with high concentrations of plasma calcitriol level increasing

substance, comprising for example isoflavones, coumestans and resorcllic acid lactones, which stimulates the synthesis of calcitriol by the animal (see for example table 1).

Fractionation of plants or plant parts, such as  
5 fractionation of grains or seeds is well known in the art. Preparing fractions enriched in phyto-estrogenic activity is for example done by milling or preparing solvent-extractions, determining the phyto-estrogenic activity of the various  
10 fractions with a method known in the art, and selecting the desired fraction. Several publications (i.e. Reinli, K. and Block, G., Nutr. Cancer 26:123-148; 1996) inform about the phyto-estrogen content in foods and fractions derived thereof.

The invention further provides a method according to the invention whereby also phytate degradation capacity of said  
15 animal is increased. It is nowadays, mainly for environmental reasons, preferred that manure derived from animals contains as little minerals as possible. In this respect, phosphate is seen by many as a mineral which presence in manure should be reduced. However, animal food, especially of plant origin,  
20 contains a fair and sometimes large amount of bound phosphate, i.e phytates such as inositol phosphate (IP-6), that is not or only little degradable to the animal, and thus leaves the digestive tract relative undigested, thereby polluting the environment. Animals of various origin are more-or-less capable  
25 of degrading phytate phosphorus, and often to animal food phytases are added to help degradation. However, said phytases are generally of recombinant nature, being enzymes derived from recombinant micro-organisms. It has even been suggested to provide transgenic or recombinant plants for incorporation in  
30 animal food, said transgenic plants having been provided with a recombinant phytase that enables better phytate degradation. However, as much as general consumers are these days wary of dietary antibiotic use, wariness exists related to use of recombinat products, thereby creating a market for so-called  
35 "natural" products.

It has also been observed that calcitriol can be helpful in degrading phyates, for example, Edwards (J. Nutr. 123:567-577, 1993) demonstrated that the degradation of IP-6 in broilers could be improved by dietary supplementation with calcitriol. As for example can be seen from figure 2 and table 2, the invention provides a method to increase phytate degradation, thereby reducing phosphate content of manure.

The invention also provides use of a dietary substance capable of increasing plasma calcitriol levels for the preparation of a supplement for an animal food suitable for increasing immune competence and/or phytate degradation of said animal. Said animal can be selected from the group of birds, fish and mammals, such as humans and ruminants who also will benefit from increased immune competence.

However, in a preferred embodiment, the invention provides use of said dietary substance for the preparation of poultry food or pig food. Poultry and pig husbandry is most under siege for both its dietary antibiotic use and its manure production, notably because with these animals genetic selection for production traits has taken great strides, thereby compromising other traits such as immune competence or phytate degradation. It is of course preferred when said substance is capable of inducing calcitriol synthesis.

The invention also provides a dietary substance or supplement for an animal food suitable for increasing immune competence of said animal comprising a substance capable of increasing plasma calcitriol levels, preferably wherein said substance is capable of inducing calcitriol synthesis. In a preferred embodiment, said substance comprises a phyto-estrogen, such as for example can be found in isolated fractions of grains and leguminous seeds, preferably soy.

The invention also provides an animal food comprising a supplement according to the invention or otherwise enriched for a substance capable of increasing plasma calcitriol levels or inducing calcitriol synthesis. Such a food is suitable feed for

all animals where raising immune competence is desired but is particularly preferred for poultry or pigs.

The invention also provides a method for increasing immune competence and/or phytate degradation capacity of an animal comprising feeding said animal a food comprising a supplement according to the invention or otherwise enriched for a substance capable of increasing plasma calcitriol levels or inducing calcitriol synthesis, preferably wherein said animal is selected from the group consisting of poultry or pigs. Use of such a method as provided by the invention is particularly useful for reducing or abolishing dietary antibiotic intake by a food animal or for reducing pospahte content of manure. For example, broilers or fattening pigs, but also reproductive, milk producing or egg laying animals, can be reared and kept under circumstances of high intrinsic immune competence, without having to resort to the excessive (or in the future even forbidden) dietary use of antibiotics or other "growth additives" that seems to be common place in traditional animal husbandry.

The invention also provides an animal having been fed a food according to the invention, preferably to partly or fully replace food supplemented with dietary antibiotics or to avoid eating recombinant products. In a preferred embodiment, the invention provides an animal which has been exclusively, or at least mainly been fed a food as provided by the invention, at least in its productive phase, such as in its fattening period or egg laying or milk producing phase in its life. Such an animal has been fed more "naturally" than under the common circumstances of high dietary antibiotics use, and can have been kept away from eating "non-natural", recombinant foods, and for example still have high resistance against disease and have high phytate degradation capacity.

The invention also provides an animal product, such as meat, milk or eggs, or, albeit not intended for human consumption, manure derived from an animal fed according to the invention. Said, more "natural", food products contain no, or

only insignificant levels of (at least dietary) antibiotic residues, especially from avoiding the use of dietary antibiotics. The invention also provides manure derived from an animal fed according to the invention, said manure (generally  
5 containing no dietary antibiotic derivatives) is containing generally less phosphate than manure derived from animals wherein phytate degradation capacity has not been increased.

Table 1. The content of calcitriol in bloodplasma ( $10^{-12}$  g/ml) of broiler chickens related to the consumption of different feedstuffs (g/kg body weight/day) containing different levels of isoflavones (mg/kg body weight/day)

	Daily intake		Calcitriol in bloodplasma
	Feedstuff	Isoflavones	
	g/kg body weight/day	mg/kg body weight/day	$10^{-12}$ g/ml
Defatted soybeanmeal	36	60	172
Peas	51	4	72
Maize	81	1	81

Table 2. Degradation of inositolphosphates (IP-6) on % in relation to the consumption of different feedstuffs in g/kg body weight/day containing different levels of isoflavones (mg/kg body weight/day)

	Daily intake		Degradation of IP-6 (%)
	Feedstuff	Isoflavones	
	g/kg body weight/day	mg/kg body weight/day	
Lupines	45	160	68,2
Defatted soybeanmeal	36	60	49,7
Fullfal soybeans	51	90	53,9
Peas	51	4	41,8
Sunflowerseed meal	35	1	38,2

Figure legends.

Figure 1.

5 Broiler chickens were fed a diet supplemented with (●) or  
without (○) a solvent-extracted soy bean derived substance  
(defatted soy bean meal) capable of increasing plasma  
calcitriol levels. At days 7 and 29, chickens were immunised  
with KLH-TNP, and at days 11, 14, 17, 21, 29, 33, 35, 38 and 42  
10 IgM directed against TNP was measured in ELISA.

Figure 2

Degradation of inositol phosphates (IP-6) in broiler chickens  
15 in % related to different levels of consumed calcitriol  
(1.25(OH)<sub>2</sub>D<sub>3</sub>)



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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/NL00/00199 <b>(22) International Filing Date:</b> 24 March 2000 (24.03.00) <b>(30) Priority Data:</b> 99200916.7 24 March 1999 (24.03.99) EP <b>(71) Applicant (for all designated States except US):</b> STICHTING DIENST LANDBOUWKUNDIG ONDERZOEK [NL/NL]; Bornsesteeg 53, NL-6708 PD Wageningen (NL). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> VAN DER KLIS, Jan, Dirk [NL/NL]; Klaproosdreef 13, NL-8255 JP Swifterband (NL). SCHEELE, Cornelis, Willem [NL/NL]; Wisselseweg 38, NL-8162 RN Epe (NL). <b>(74) Agent:</b> OTTEVANGERS, S., U.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).		<b>(81) Designated States:</b> AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> INCREASING PLASMA CALCITRIOL LEVELS		
<b>(57) Abstract</b>  The invention relates to animal food and supplements used therein. The invention provides a method for preparing an animal food suitable for increasing immune competence and/or phytate degradation competence of said animal comprising supplementing or enriching said food with a substance capable of increasing plasma calcitriol levels. Use of such a method as provided by the invention is particularly useful for reducing or abolishing dietary antibiotic intake by a food animal. For example, broilers or fattening pigs, but also reproductive or egg laying animals, can be reared and kept under circumstances of high intrinsic immune competence, without having to resort to the excessive or forbidden dietary use of antibiotics or other "growth additives" that seems to be common place in traditional animal husbandry.		

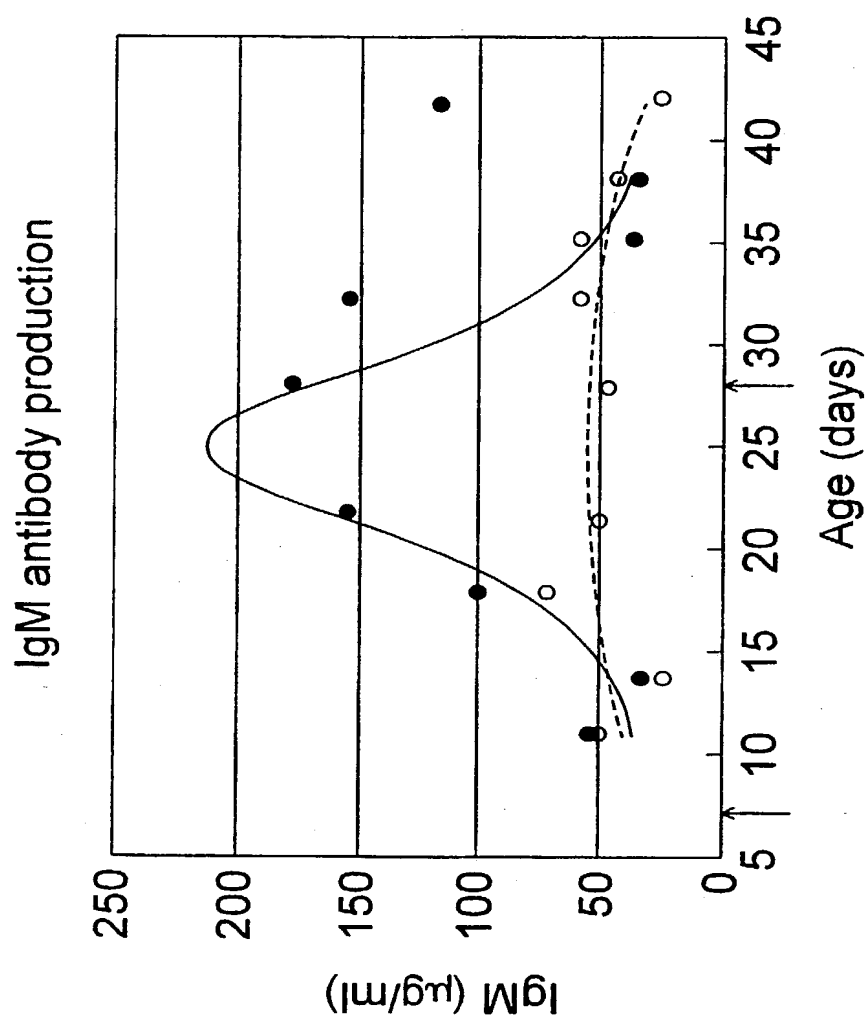


Fig. 1

The IgM antibody production in broiler chickens after immunisation with KLH-TNP at 7 and 28 days of age (indicated by arrows) with (●) and without (○) 1.25 DHCC as an adjuvant.

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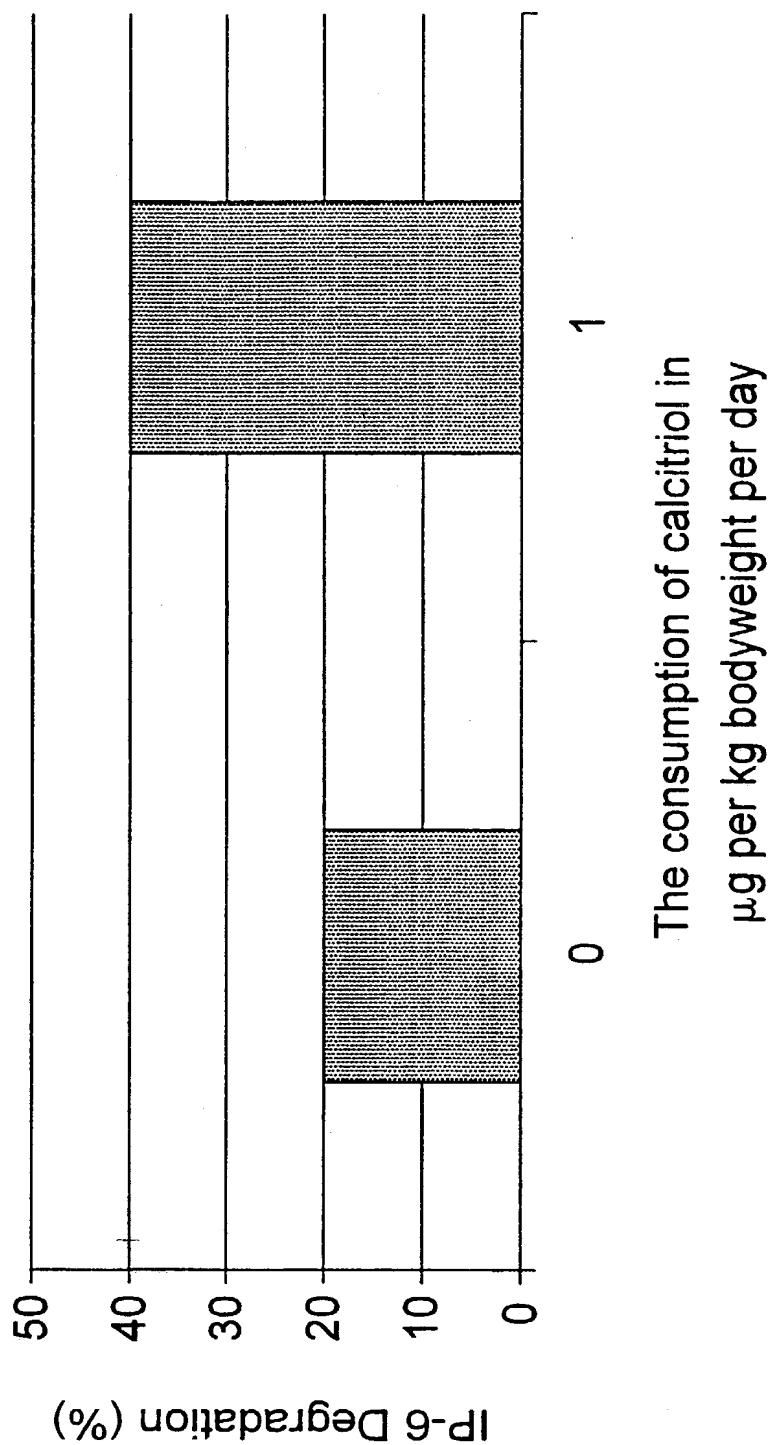


Fig. 2

Attorney Docket No. 2183-5091US

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As an inventor named below or on any attached continuation page, I hereby declare that:  
My residence, post office address and citizenship are as stated next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below)  
or an original, first and joint inventor (if plural names are listed below) of the subject matter  
which is claimed and for which a patent is sought on the invention entitled **INCREASING  
PLASMA CALCITRIOL LEVELS**, the specification of which (check one):

- ☐ is attached hereto.  
☒ as filed on December 11, 2001, as United States application serial no. 10/018,390  
 and was amended on \_\_\_\_\_.  
☐ as filed on \_\_\_\_\_ as PCT international application no. \_\_\_\_\_ and  
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Prior foreign/PCT application(s):

99200916.7	EP	24-03-1999	Priority <b>X</b>	Claimed
(number)	(country)	(day/month/year filed)	Yes	No
_____	_____	_____	Yes	No
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Invention Title: INCREASING PLASMA CALCITRIOL LEVELS

between the filing date of such prior application and the national or PCT international filing date of this application:

_____	_____	_____
(application serial no.)	(filing date)	(status—pending, patented or abandoned)
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I hereby appoint the following Registered Practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

David V. Trask, Reg. No. 22,012  
 Laurence B. Bond, Reg. No. 30,549  
 James R. Duzan, Reg. No. 28,393  
 Allen C. Turner, Reg. No. 33,041  
 Kent S. Burningham, Reg. No. 30,453  
 Kevin K. Johanson, Reg. No. 38,506  
 Devin R. Jensen, Reg. No. 44,805  
 David L. Stott, Reg. No. 43,937  
 Bretton L. Crockett, Reg. No. 44,632  
 Bradley B. Jensen, Reg. No. 46,801  
 Greg T. Warder, Reg. No. 50,208  
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William S. Britt, Reg. No. 20,969  
 Joseph A. Walkowski, Reg. No. 28,765  
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 Krista Weber Powell, Reg. No. 47,867  
 Shawn G. Hansen, Reg. No. 42,627  
 Tawni L. Wilhelm, Reg. No. 47,456  
 Andrew F. Nilles, Reg. No. 47,825  
 Katherine A. Harner, Reg. No. 47,628

23

Address all correspondence to: Allen C. Turner, telephone no. (801) 532-1922.

TRASKBRITT, PC  
P.O. Box 2550  
Salt Lake City, Utah 84110

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Invention Title: INCREASING PLASMA CALCITRIOL LEVELS

Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first joint inventor: Jan Dirk van der Klis

Inventor's signature

Date

25/6 2002

Residence: Swifterbant, The Netherlands

Citizenship: The Netherlands

Post Office Address: Klaproosdreef 13, Swifterbant, 8255 JP, The Netherlands

Full name of second joint inventor: Cornelis Willem Scheele

Inventor's signature

Date

27/6 - 2002

Residence: Epe, The Netherlands

Citizenship: The Netherlands

Post Office Address: Wisselseweg 38, Epe, 8162 RN, The Netherlands

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